

# Coenzyme Q 10 Abrogates Flutamide-Induced Hepatotoxicity in Albino Rats

Elias Adikwu, Nelson Clemente Ebinyo, Harold Aagbadabina

## ABSTRACT

**Aim:** The efficacy and therapeutic benefit of flutamide (FLU) can be clinically limited by the occurrence of hepatotoxicity which may progress to liver failure. This study, therefore, evaluated the protective effect of coenzyme Q 10 (COQ 10) against FLU-induced liver toxicity in albino rats.

**Materials and Methods:** Forty-five adult male albino rats of average weight 250 g were randomized into groups and treated intraperitoneally with COQ 10; 50 mg/kg + FLU 200 mg/kg, COQ 10; 100 mg/kg + FLU 200 mg/kg and COQ 10; 200 mg/kg + FLU 200 mg/kg daily for 7 days. On the 8<sup>th</sup> day, rats were sacrificed, serum samples were extracted from blood and liver was excised and evaluated for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin (TB), and conjugated bilirubin (CB). Liver was evaluated for oxidative stress markers and pathologic changes.

**Results:** Serum and liver AST, ALT, ALP, AST, GGT, LDH, TB, and CB levels were significantly ( $P < 0.001$ ) increased in FLU-treated rats when compared to placebo control. Liver superoxide dismutase, catalase, glutathione, and glutathione peroxidase were decreased, whereas malondialdehyde levels were increased significantly ( $P < 0.001$ ) in FLU-treated rats when compared to placebo control. The liver of FLU-treated rats showed hepatocyte necrosis. However, FLU-induced liver toxicity was significantly abrogated in a dose-dependent manner in COQ 10; 50 mg/kg ( $P < 0.05$ ), COQ 10; 100 mg/kg ( $P < 0.01$ ); and COQ 10; 200 mg/kg ( $P < 0.001$ ) pre-treated rats when compared to FLU-treated rats.

**Conclusion:** This study showed that COQ 10 abrogates FLU-induced hepatotoxicity in albino rats in a dose-dependent manner. It may have clinical application in hepatotoxicity associated with FLU.

**KEY WORDS:** Antioxidant, flutamide, liver, protection, rat, toxicity.

## Introduction

Flutamide (FLU) which competitively antagonizes androgen receptor is used in combination with castration in the treatment of prostatic cancer. It competes for androgen receptor in the prostate gland with testosterone hormone and its biotransformed and active metabolite; dihydrotestosterone. Its action can inhibit the stimulatory effects of testosterone hormone and its active metabolite dihydrotestosterone on prostate cancer cells, thereby preventing the growth and spread of prostate cancer cells.<sup>[1]</sup> However, the efficacy and therapeutic benefit of FLU can be clinically limited by the occurrence of hepatotoxicity in prostate cancer

patients. Hepatotoxicity due to therapy with FLU includes acute hepatitis, which may progress to a fulminant form (acute liver failure) that may require liver transplantation. It is often characterized by elevations in liver enzymes and in some cases alterations in liver histology. The incidence of hepatotoxicity associated with FLU has been reported to be 6–9% in prostate cancer patients.<sup>[2]</sup> This instigated U.S Food and Drug Administration to ask for a black box warning on FLU label to highlight the possible development of hepatotoxicity during therapy. The precise mechanism by which FLU causes hepatotoxicity is poorly understood; however, some studies attributed it to FLU metabolites which can initiate and propagate free radical production leading to hepatic oxidative stress (OS) and consequently to lipid peroxidation (LPO) and hepatic injury.<sup>[3]</sup> Furthermore, *in-vitro* and *in-vivo* studies have associated FLU with the generation of free radicals in liver tissues.<sup>[4]</sup> Additionally, some studies suggested the involvement of mitochondrial dysfunction as indicated by altered mitochondrial

Access this article online

Quick Response Code:



Website: [www.jmsh.ac.in](http://www.jmsh.ac.in)

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria

Address for correspondence:

Dr. Elias Adikwu, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria.

E-mail: [adikwuelias@gmail.com](mailto:adikwuelias@gmail.com)

morphology, mitochondrial membrane potential loss, and ATP depletion in FLU-induced hepatotoxicity.<sup>[5]</sup>

Coenzyme Q10 (COQ10) is a biochemical compound that is endogenously produced and is present in human cells. It has a benzoquinone ring and a polyisoprenoid tail that contains 6 to 10 subunits which are species-specific and enhances its stability.<sup>[6]</sup> It is a redox-active lipophilic free radical scavenger that is present in cellular organelles such as lysosomes, mitochondria, and Golgi vesicles.<sup>[7]</sup> In mitochondria, COQ10 plays an important role in the production of energy by functioning as an electron carrier in the electron transport chain.<sup>[8]</sup> Outside the mitochondria, it functions as an essential antioxidant by scavenging free radicals and working in synergy with other antioxidants. It can abrogate the initiation and propagation of free radicals, thereby inhibiting the detrimental effects of free radicals on lipids, proteins, DNA, and the functionality of mitochondria.<sup>[9,10]</sup> Furthermore, *in-vitro* studies using animal models have reported the activities of COQ10 as an anti-inflammatory agent. Other activities of COQ 10 include modulation of the functions of the immune system<sup>[11]</sup> stabilization of membrane and the modulation of gene expression.<sup>[12]</sup> COQ10 has gained attention due to its possible therapeutic effects on certain diseases such as neurodegenerative diseases, cancer, cardiovascular diseases, and diabetes.<sup>[13]</sup> The current study, therefore, assessed whether pretreatment with COQ10 can protect against FLU-induced hepatotoxicity, which has not been evaluated.

## Materials and Methods

Forty-five adult male albino rats, weighing 250 g were sourced from the animal house of the Faculty of Pharmacy, University of Port Harcourt, Nigeria. The rats were domiciled in the animal house of the Faculty of Pharmacy, Niger Delta University, Nigeria, where the study was performed. The rats were housed at a temperature of  $23 \pm 2^\circ\text{C}$  with a cycle of 12 light/12 dark and were allowed to acclimatize for 2 weeks before the experiment with free access to standard diet and water *ad libitum*. This study was approved by the Research Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The rats were handled according to the guidelines of the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

### Dose selection and animal treatment

COQ (50 mg/kg, 100 mg/kg, and 200 mg/kg) dissolved in corn oil<sup>[14]</sup> and FLU (200 mg/kg)<sup>[15]</sup> were

used for this study. The rats were assigned randomly to nine groups (A-I) of  $n = 5$ . Groups A and B served as placebo and solvent control and were treated intraperitoneally (i.p) with water and corn oil, for 7 days respectively, whereas groups C-E were treated i.p with COQ 10 (50 mg/kg, 100 mg/kg, and 200 mg/kg) daily for 7 days respectively. Group F was treated i.p with FLU 200 mg/kg daily for 7 days. On the other hand, groups G-I were treated i.p with COQ 10; 50 mg/kg + FLU 200 mg/kg, COQ 10; 100 mg/kg + FLU 200 mg/kg and COQ 10; 200 mg/kg + FLU 200 mg/kg daily for 7 days respectively.

### Animal sacrifice

On the 8<sup>th</sup> day of the study, rats were euthanized with diethyl ether, and blood samples were drained from the heart. The blood samples were collected in a plain sample container and allowed to clot at room temperature. The serum samples were extracted from the clot by centrifugation at 3000 rpm for 15 min and used for the evaluation of biochemical parameters. Liver was harvested rinsed in ice cold saline. It was sliced, minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10%w/v) with the aid of Teflon pestle homogenizer at a speed of 2500 rpm. The clear supernatant was collected and used for the assessment of OS markers and aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH).

### Evaluation of liver function markers

Evaluations of serum and liver AST, ALT, ALP, GGT, LDH, total bilirubin (TB), and conjugated bilirubin (CB) were carried out using standard laboratory test kits according to manufacturer's specification.

### OS marker assay

Liver malondialdehyde (MDA) was assayed, according to Buege and Aust<sup>[16]</sup> whereas reduced glutathione (GSH) was assayed as reported by Sedlak and Lindsay<sup>[17]</sup> Superoxide dismutase (SOD) was determined as reported by Sun and Zigma<sup>[18]</sup> whereas catalase (CAT) was evaluated according to Sinha *et al.*, 1972.<sup>[19]</sup> Glutathione peroxidase (GPX) was determined according to Rotruck *et al.*, 1973.<sup>[20]</sup>

### Histological examination of the liver

Rats were dissected after euthanasia; liver was harvested, blotted free of blood, and fixed in 10% formalin solution for 24 h. The fixed liver tissues were dehydrated with the aid of ascending alcohol solutions then cleared in xylene. Tissues were

embedded in paraffin blocks and sectioned with the aid of a microtome. 5  $\mu$ m thick sections of the tissues were stained with hematoxylin and eosin. Stained sections were examined with the aid of a light microscope, and relevant sections were photographed.

### Statistical analysis

Results were statistically evaluated by one-way ANOVA using SPSS 20.00, followed by Tukey's *post hoc* test. Results are expressed as the mean  $\pm$  standard error of mean.  $P < 0.05$ , 0.01, and 0.001 were considered significant.

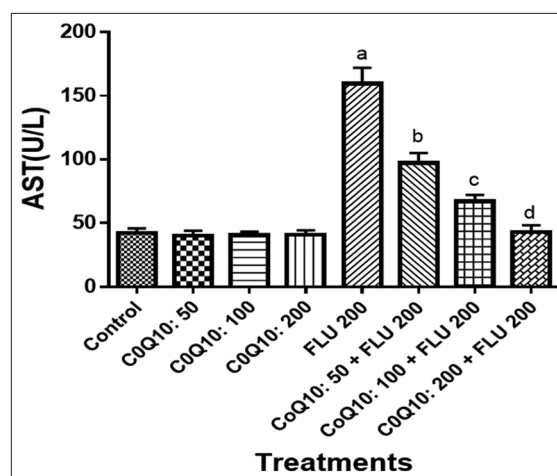
## Results

### Effects on COQ 10 on liver function markers of FLU-treated rats

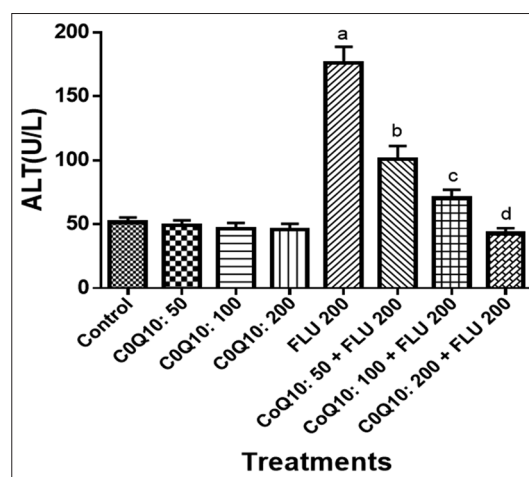
The administration of COQ 10 (50 mg/kg, 100 mg/kg, and 200 mg/kg) did not produce significant ( $P > 0.05$ ) effects on serum AST, ALT, ALP, GGT, LDH, TB, and CB levels when compared to placebo control (Figures 1-7). However, the aforementioned parameters were significantly ( $P < 0.001$ ) elevated in rats treated with FLU 200 mg/kg when compared to control. Interestingly, the serum levels of AST, ALT, ALP, GGT, LDH, TB, and CB were decreased in a dose-dependent fashion in rats treated with COQ 10; 50 mg/kg + FLU 200 mg/kg ( $P < 0.05$ ), COQ 10; 100 mg/kg + FLU 200 mg/kg ( $P < 0.01$ ), and COQ 10; 200 mg/kg + FLU 200 mg/kg ( $P < 0.001$ ) when compared to rats treated with FLU 200 mg/kg (Figures 1-7). Normal ( $P > 0.05$ ) levels of AST, ALT, ALP, GGT, and LDH were obtained in the liver of COQ 10 (50, 100, and 200 mg/kg) treated rats when compared to control (Table 1). However, the above parameters were significantly ( $P < 0.001$ ) increased in rats treated with FLU 200 mg/kg when compared to control (Table 1). It is noteworthy that AST, ALT, ALP, GGT, and LDH levels were significantly decreased in a dose-dependent manner in COQ 10; 50 mg/kg + FLU 20 mg/kg ( $P < 0.05$ ), COQ 10; 100 mg/kg + FLU 200 mg/kg ( $P < 0.01$ ), and COQ 10; 200 mg/kg + FLU 200 mg/kg ( $P < 0.001$ ) treated rats when compared to rats treated with FLU 200 mg/kg (Table 1).

### Effects of COQ 10 on liver OS indices and histology of FLU-treated rats

Furthermore, the liver levels of SOD, CAT, GSH, GPX, and MDA were normal ( $P > 0.05$ ) in rats administered with COQ 10 (50 mg/kg, 100 mg/kg, and 200 mg/kg) when compared to placebo control (Table 2). On the other hand, the liver levels of SOD, CAT, GSH, and GPX were significantly ( $P < 0.001$ ) decreased whereas

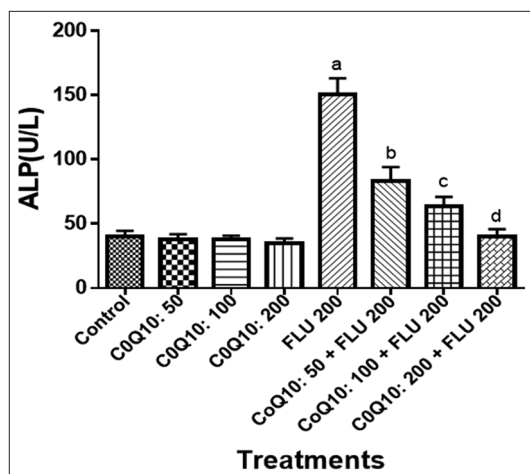


**Figure 1:** Effect of coenzyme Q10 on serum aspartate aminotransferase of flutamide-treated albino rats. AST: Aspartate Aminotransferase CoQ10: Coenzyme Q10, FLU: Flutamide.  $n = 5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU

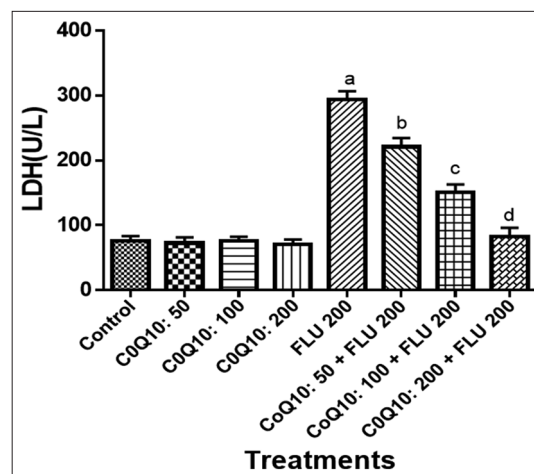


**Figure 2:** Effect of coenzyme Q10 on serum alanine aminotransferase of flutamide-treated albino rats. ALT: Alanine Aminotransferase, CoQ10: Coenzyme Q10, FLU: Flutamide.  $n = 5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU

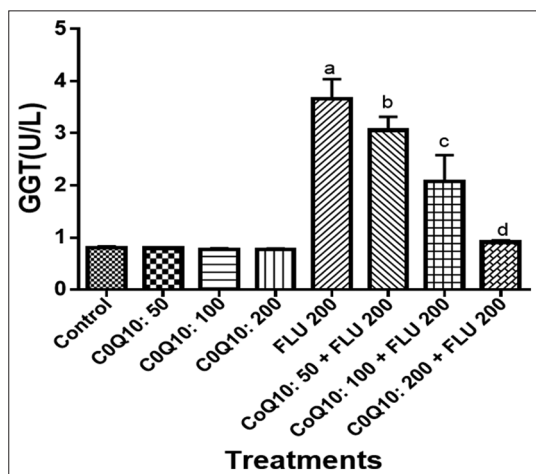
MDA levels were significantly ( $P < 0.001$ ) increased in rats treated with FLU 200 mg/kg when compared to control (Table 2). However, the liver levels of SOD, CAT, GSH, and GPX were significantly increased, whereas MDA levels were significantly decreased in



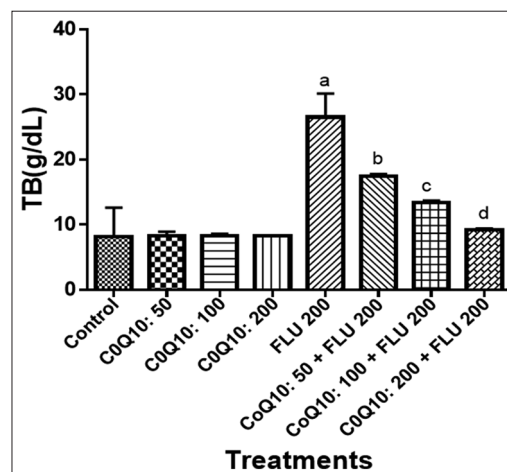
**Figure 3:** Effect of coenzyme Q10 on serum alkaline phosphatase of flutamide-treated albino rats. ALP: Alkaline Phosphate, COQ10: Coenzyme Q10, FLU: Flutamide,  $n=5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU



**Figure 5:** Effect of coenzyme Q10 on serum lactate dehydrogenase of flutamide-treated albino rats. LDH: Lactate Dehydrogenase, COQ10: Coenzyme Q10, FLU: Flutamide.  $n = 5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU



**Figure 4:** Effect of coenzyme Q10 on serum gamma-glutamyl transferase of flutamide-treated albino rats. GGT: Gamma Glutamyl Transferase, COQ10: Coenzyme Q10, FLU: Flutamide.  $n = 5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU



**Figure 6:** Effect of coenzyme Q10 on serum total bilirubin of flutamide-treated albino rats. TB: Total Bilirubin, CoQ10: Coenzyme Q10, FLU: Flutamide.  $n=5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU

a dose-dependent manner in rats treated with COQ 10; 50 mg/kg + FLU 200 mg/kg ( $P < 0.05$ ), COQ 10; 100 mg/kg + FLU 200 mg/kg ( $P < 0.01$ ), and COQ 10; 200 mg/kg + FLU 200 mg/kg ( $P < 0.001$ ) when compared to FLU 200 mg/kg treated rats (Table 2). The control rat

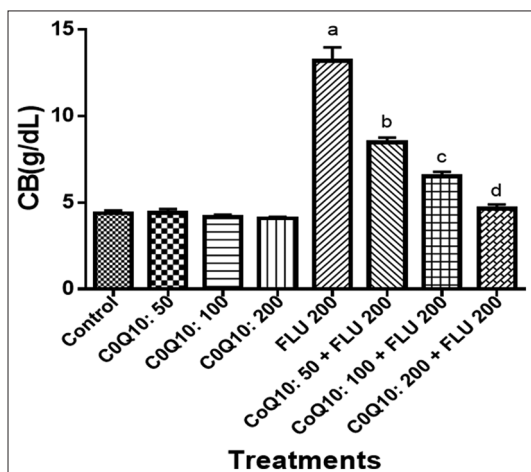
showed normal hepatocytes whereas rats treated with FLU 200 mg/kg showed hepatocyte necrosis (Figure 8a and b). On the other hand, rats treated with COQ 10; 50 mg/kg + FLU 200 mg/kg showed areas of lipid necroses (Figure 8c). However, rats treated with COQ



10; 100 mg/kg + FLU 200 mg/kg (Figure 8d) and COQ 10; and 200 mg/kg + FLU 200 mg/kg showed normal liver histology (Figure 8e).

## Discussion

The surge in the clinical use of FLU in the fight against prostate cancer is quite overwhelming with positive therapeutic outcome. The pains and daunting challenges that have bedeviled the scourge of prostate cancer have been nip in the bud by FLU. However, the hepatotoxic consequence that characterizes the clinical application of FLU could instigate a rethink and reassessment of risk/benefit



**Figure 7:** Effect of coenzyme Q10 on serum conjugated bilirubin of flutamide-treated albino rats. CB: Conjugated Bilirubin, CoQ10: Coenzyme Q10, FLU: Flutamide.  $n = 5$ , data expressed as Mean  $\pm$  SEM. <sup>a</sup>Significant ( $P < 0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P < 0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P < 0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P < 0.001$ ) difference when compared to FLU

ration in some quarters.<sup>[21]</sup> Furthermore, the limited or the relatively available treatment options for hepatic perturbation caused by FLU is a daunting challenge. The present study, therefore, deem it necessary to open up a new frontier by evaluating whether COQ10 can safeguard the liver of FLU intoxicated rats. In both clinical and experimental settings, ALT, ALP, AST, GGT, LDH, and bilirubin are among the determinants employed to ascertain the nature and extent of hepatic perturbations caused by xenobiotics. ALT is highly precise in monitoring hepatocellular status, whereas AST is a basic and sensitive yardstick for hepatic mitochondrial perturbation, especially around the centrilobular areas of the liver. In a scenario of compromised hepatic integrity, the aforementioned hepatic function markers are often elevated above the established clinical benchmark.<sup>[22]</sup> The current study observed functional compromise in the liver of FLU- treated rats as evidenced by elevated serum and liver levels of ALT, ALP, AST, GGT, LDH, CB, and TB. This observation is consistent with reported altered serum hepatic function markers due to treatment with FLU.<sup>[23]</sup> However, FLU-induced perturbation in hepatic function was abrogated in a dose-dependent fashion by pretreatment with COQ 10 as evidenced by the restored serum and liver levels of the aforementioned parameters.

OS through free radical generation is often regulated by the activities of SOD, CAT, GSH, GPX and other antioxidants thereby limiting the potential damage that may arise from their actions.<sup>[24]</sup> SOD catalyzes the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide. Furthermore, it prevents peroxynitrite formation, mitochondrial,

**Table 1: Effect of COQ 10 on liver tissue biochemical parameters of flutamide-treated albino rats**

Treatment (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Control (Placebo)	234.9 $\pm$ 16.7	223.8 $\pm$ 15.2	225.5 $\pm$ 15.9	22.6 $\pm$ 3.24	225.6 $\pm$ 14.9
COQ10: 50	227.7 $\pm$ 12.0	225.5 $\pm$ 17.9	230.0 $\pm$ 17.0	23.3 $\pm$ 2.44	229.4 $\pm$ 13.8
COQ10: 100	221.5 $\pm$ 13.9	228.4 $\pm$ 16.2	224.4 $\pm$ 16.7	23.0 $\pm$ 2.58	225.7 $\pm$ 14.3
COQ10: 200	228.0 $\pm$ 13.36	225.3 $\pm$ 15.9	214.1 $\pm$ 15.0	21.7 $\pm$ 2.25	220.1 $\pm$ 13.2
FLU 200	922.9 $\pm$ 21.9 <sup>a</sup>	883.9 $\pm$ 18.3 <sup>a</sup>	884.2 $\pm$ 20.0 <sup>a</sup>	97.0 $\pm$ 8.87 <sup>a</sup>	807.0 $\pm$ 22.0 <sup>a</sup>
COQ10: 50+FLU 200	579.9 $\pm$ 13.34 <sup>b</sup>	562.3 $\pm$ 16.5 <sup>b</sup>	557.7 $\pm$ 20.5 <sup>b</sup>	65.8 $\pm$ 6.27 <sup>b</sup>	537.6 $\pm$ 14.9 <sup>b</sup>
COQ10: 100+FLU 200	360.2 $\pm$ 18.2 <sup>c</sup>	337.4 $\pm$ 18.0 <sup>c</sup>	346.3 $\pm$ 15.6 <sup>c</sup>	45.9 $\pm$ 3.95 <sup>c</sup>	333.8 $\pm$ 12.5 <sup>c</sup>
COQ10: 200+FLU 200	238.0 $\pm$ 20.7 <sup>d</sup>	227.4 $\pm$ 15.7 <sup>d</sup>	235.8 $\pm$ 13.5 <sup>d</sup>	26.8 $\pm$ 3.06 <sup>d</sup>	246.7 $\pm$ 13.1 <sup>d</sup>

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphate, GGT: Gamma-Glutamyl Transferase, TB: Total Bilirubin, CB: Conjugated Bilirubin, LDH: Lactate Dehydrogenase, CoQ10: Coenzyme Q10, FLU: Flutamide.  $n=5$ , data expressed as Mean $\pm$ SEM. <sup>a</sup>Significant ( $P<0.001$ ) difference when compared to control. <sup>b</sup>Significant ( $P<0.05$ ) difference when compared to FLU. <sup>c</sup>Significant ( $P<0.01$ ) difference when compared to FLU. <sup>d</sup>Significant ( $P<0.001$ ) difference when compared to FLU

**Table 2: Effect of coenzyme Q10 on liver oxidative stress parameters of flutamide-treated albino rats**

Treatment (mg/kg)	SOD (u/mg protein)	CAT (u/mg protein)	GSH (μg/mg protein)	GPX (u/mg protein)	MDA (nmol/mg protein)
Control	23.9±2.49	42.2±1.24	11.0±0.44	18.8±1.55	0.14±0.01
COQ10: 50	24.4±2.74	43.0±3.94	11.3±0.51	18.7±1.79	0.13±0.01
COQ10: 100	26.0±2.51	44.7±3.75	11.6±0.28	20.1±1.53	0.11±0.02
COQ10: 200	26.5±2.50	46.9±4.47	12.3±0.17	19.3±1.93	0.12±0.01
FLU 200	8.20±0.56 <sup>a</sup>	14.6±1.53 <sup>a</sup>	4.09±0.16 <sup>a</sup>	5.69±0.19 <sup>a</sup>	0.42±0.02 <sup>a</sup>
CoQ10: 50+FLU 200	12.1±1.37 <sup>b</sup>	24.1±1.49 <sup>b</sup>	6.34±0.11 <sup>b</sup>	7.79±0.07 <sup>b</sup>	0.27±0.01 <sup>b</sup>
CoQ10: 100+FLU 200	17.6±1.45 <sup>c</sup>	32.6±2.69 <sup>c</sup>	8.06±0.22 <sup>c</sup>	11.0±0.20 <sup>c</sup>	0.20±0.01 <sup>c</sup>
COQ10: 200+FLU 200	22.1±2.44 <sup>d</sup>	39.0±3.45 <sup>d</sup>	10.2±0.23 <sup>d</sup>	16.0±1.55 <sup>d</sup>	0.16±0.01 <sup>d</sup>

MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase, SOD: Superoxide Dismutase, GPX: Glutathione peroxidase, COQ10: Coenzyme Q10, FLU: Flutamide. *n*=5, data expressed as Mean±SEM. <sup>a</sup>Significant (*P*<0.001) difference when compared to control. <sup>b</sup>Significant (*P*<0.05) difference when compared to FLU. <sup>c</sup>Significant (*P*<0.01) difference when compared to FLU.

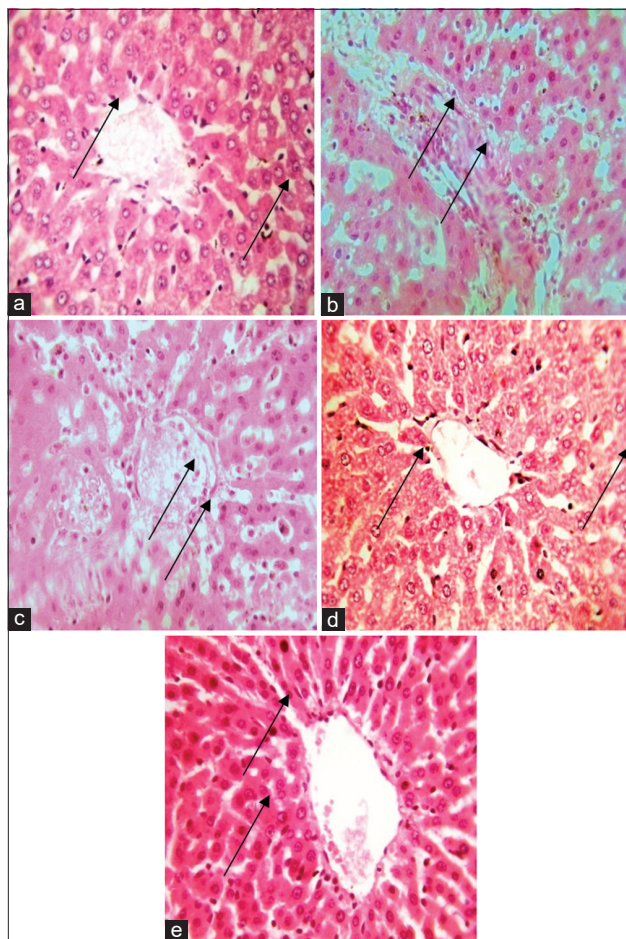
<sup>d</sup>Significant (*P*<0.001) difference when compared to FLU

and endothelial dysfunctions that may be associated with nitric oxide.<sup>[25]</sup> CAT scavenges free radicals and also protects cells against damage caused by lipoperoxides or hydroperoxides<sup>[26]</sup> whereas GSH directly or indirectly scavenges free radicals.<sup>[27]</sup> However, excess or uncontrollable activities of free radicals can overwhelm the opposing functions of SOD, CAT, GSH, and GPX, thereby culminating in OS and tissue damage. The current study observed hepatic OS marked by alterations in antioxidant status in FLU-intoxicated rats as evidenced by decreases in hepatic SOD, CAT, GSH, and GPX content. This finding is consistent with decreases in hepatic antioxidant status reported in FLU-intoxicated rats.<sup>[28]</sup> In contrast, hepatic antioxidant levels were restored in a dose-dependent manner in rats pretreated with COQ 10 characterized by vivid increases in SOD, CAT, GSH, and GPX levels.

LPO which plays an important role in pathological processes is a catalytic free radical-induced oxidative deterioration of polyunsaturated fatty acids in cells leading to lipid hydroperoxides formation and subsequently ketones, aldehydes, alkanals, alkenals, and MDA formations.<sup>[29]</sup> The extent and nature of injury caused by LPO can be measured by conjugated dienes, MDA, 4-hydroxynonenal, and others byproducts. However, MDA has been primarily used to evaluate and validate the occurrence of LPO.<sup>[30]</sup> The current study observed marked hepatic LPO characterized by higher MDA levels in FLU-treated rats. This observation is consistent with the reported increase in MDA level due to FLU intoxication.<sup>[31]</sup> Interestingly, the induction of LPO by FLU was abrogated in COQ10 supplemented rats in a dose-dependent manner.

Histopathology is the microscopic study of diseased tissues. It is an important investigative medical tool that is based on the study of human or animal histology with the aid of a microscope. It allows visualization of tissue and cell microscopic features and recognizes specific microscopic structural changes caused by diseases. It basically compares diseased or experimentally altered tissues with matching sample from healthy or control counterparts.<sup>[32]</sup> The histological assessment of the liver of rats administered with FLU showed altered liver morphology accompanied by hepatocyte necrosis. The observation is an agreement with altered hepatic morphology reported in previous studies.<sup>[33]</sup> It is quite fascinating that FLU-induced hepatocyte necrosis was abrogated in rats supplemented with COQ10 in a dose-dependent manner.

The mechanism by which FLU causes hepatotoxicity remains unclear; however, studies have associated it with its hepatic biotransformed product 2-hydroxy FLU (OH-FLU). *In-vitro* studies have confirmed the toxic effect of OH-FLU on cultured rat hepatocytes. Furthermore, 5-amino-2-nitrobenzotrifluoride (FLU-1) a metabolite obtained from FLU hydroxylation may play a significant role in FLU-induced hepatotoxicity. Studies further suggested that FLU-induced hepatotoxicity can be associated with hydrolysis operated by an arylacetamide deacetylase on FLU.<sup>[34]</sup> Furthermore, the net effects of the aforementioned metabolites can be OS, inflammation, and mitochondria dysfunction.<sup>[35,36]</sup> In the current study, the ability of COQ 10 to safeguard the liver from FLU-intoxication can be attributed to its antioxidant and anti-inflammatory effects. Due to the antioxidant effect of COQ10, it might have



**Figure 8:** Micrographs of the liver of control rat and rats in the experimental groups (a-e). Control liver showing normal histology (a). Liver of rat treated with 200 mg/kg of flutamide (FLU) showing hepatocyte necrosis (b). Liver of rat treated with 50 mg/kg of Coenzyme Q10 (COQ 10) + 200 mg/kg of FLU showing areas of lipoid necrosis (c). Liver of rats treated with 100 mg/kg of COQ 10 + 200 mg/kg of FLU showing normal hepatocytes (d). Furthermore, liver of rats treated with 200 mg/kg of COQ 10 + 200 mg/kg of FLU showing normal hepatocytes (e)

scavenged free radicals and protected membrane phospholipids, mitochondrial membrane protein from OS and LPO-induced damage.<sup>[37]</sup> Furthermore, due to its anti-inflammatory effect, it might have inhibited the production of pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  and Interleukin-1 $\beta$  that are associated with hepatotoxicity. In addition, COQ10 might have stimulated hepatic cell growth, antioxidant regeneration, and inhibited hepatic cell death.<sup>[38]</sup>

## Conclusion

In the current study, supplementation with COQ 10 abrogated FLU-induced hepatotoxicity in albino rats in

a dose-dependent fashion. COQ 10 may have clinical application in hepatotoxicity associated with FLU.

## Acknowledgments

The authors appreciate Dr. Obuma Yibala of the Department of Medical Laboratory Sciences, Niger Delta University, Nigeria, for the histological study. Also, we appreciate Mr. Obi Cosmos of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria, for animal handling.

## References

1. Elks J. The Dictionary of Drugs: Chemical Data, Structures and Bibliographies. Berlin, Germany: Springer Science Business Media; 2014. p. 368.
2. Cetin M, Demirci D, Unal A, Altinbaş M, Güven M, Unlühizarci K, *et al.* Frequency of flutamide induced hepatotoxicity in patients with prostate carcinoma. *Hum Exp Toxicol* 1999;18:137-40.
3. Matsuzaki Y, Nagai D, Ichimura E, Goda R, Tomura A, Doi M, *et al.* Metabolism and hepatic toxicity of flutamide in cytochrome P450 1A2 knockout SV129 mice. *J Gastroenterol* 2006;41:231-9.
4. Al Maruf A, O'Brien P. Flutamide-induced cytotoxicity and oxidative stress in an *in vitro* rat hepatocyte system. *Oxid Med Cell Longev* 2014;2014:398285.
5. Zhang L, Guo J, Zhang Q, Zhou W, Li J, Yin J, *et al.* Flutamide induces hepatic cell death and mitochondrial dysfunction via inhibition of nrf2-mediated heme oxygenase-1. *Oxid Med Cell Longev* 2018;2018:8017073.
6. López-Lluch G, Rodríguez-Aguilera JC, Santos-Ocaña C, Navas P. Is coenzyme Q a key factor in aging? *Mech Ageing Dev* 2010;131:225-35.
7. Laredj LN, Licitra F, Puccio HM. The molecular genetics of coenzyme Q biosynthesis in health and disease. *Biochimie* 2014;100:78-87.
8. Molyneux SL, Young JM, Florkowski CM, Lever M, George PM. Coenzyme Q10: Is there a clinical role and a case for measurement? *Clin Biochem Rev* 2008;29:71-82.
9. Lance J, McCabe S, Clancy RL, Pierce J. Coenzyme Q10 a therapeutic agent. *Medsurg Nurs* 2012;21:367-71.
10. Sohet FM, Delzenne NM. Is there a place for coenzyme Q in the management of metabolic disorders associated with obesity? *Nutr Rev* 2012;70:631-41.
11. Alam MA, Rahman MM. Mitochondrial dysfunction in obesity: Potential benefit and mechanism of co-enzyme Q10 supplementation in metabolic syndrome. *J Diabetes Metab Disord* 2014;13:60.
12. Neergheen V, Chalasani A, Wainwright L, Yubero D, Montero R, Artuch R, *et al.* Coenzyme Q10 in the treatment of mitochondrial disease. *J Inborn Errors Metab Screen* 2017;5:1-8.
13. Crozier A, Borges G. (Poly)phenolic constituents and the beneficial effects of moderate red wine consumption. *J Wine Res* 2011;22:131-4.
14. Song MH, Kim HN, Lim Y, Jang IS. Effects of coenzyme Q<sub>10</sub> on the antioxidant system in SD rats exposed to lipopolysaccharide-induced toxicity. *Lab Anim Res* 2017;33:24-31.
15. Imperato-McGinley J, Sanchez RS, Spencer JR, Yee B, Vaughan ED. Comparison of the effects of the 5 alpha-reductase



- inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: Dose-response studies. *Endocrinology* 1992;131:1149-56.
16. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978;52:302-10.
  17. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968;25:192-205.
  18. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Anal Biochem* 1978;90:81-9.
  19. Sinha AK. Colorimetric assay of catalase. *Anal Biochem* 1972;47:389-94.
  20. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, *et al.* Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973; 179:588-90.
  21. de Amorim MF, de Amorim WP, Duques P, de Amorim PD, de Vasconcelos JR. Flutamide-induced hepatotoxicity during treatment of acne a case report. *Bras Dermatol* 2005;80:381-4.
  22. Wang Y, Tang C, Zhang H. Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from *Carthamus tinctorius* L. On CCl<sub>4</sub>-induced oxidative liver injury in mice. *J Food Drug Anal* 2015;23:310-7.
  23. Bruni V, Peruzzi E, Dei M, Nannini S, Seravalli V, Sisti G, *et al.* Hepatotoxicity with low and ultralow-dose flutamide: A surveillance study on 203 hyperandrogenic young females. *Fertil Steril* 2012;98:1047-52.
  24. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol* 2018;217:1915-28.
  25. Fukai T, Ushio-Fukai M. Superoxide dismutases: Role in redox signaling, vascular function, and diseases. *Antioxid Redox Signal* 2011;15:1583-606.
  26. Pereira MD, Eleutherio EC, Panek AD. Acquisition of tolerance against oxidative damage in *saccharomyces cerevisiae*. *BMC Microbiol* 2001;1:11.
  27. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, *et al.* Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* 2015;6:183-97.
  28. Mazhar FM, Kadry SM, Abd-Allah HF, Sabry HA, Saleh AS. Protective effect of morin against flutamide-induced hepatotoxicity in mal Wister rats. *Egypt J Hosp Med* 2017;67:336-43.
  29. Dianzai M, Barrera G. In: Alvarez S, Evelson P, editors. *Pathology and Physiology of Lipid Peroxidation and its Carbonyl Products, Free Radical Pathology*. Kerala, India: Transworld Research Network; 2008. p. 19-38.
  30. Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC. Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. *Quim Nova* 2009;32:169-74.
  31. Ray SD, Chowdhury P, Ray S. Evaluation of protective effect of reduced glutathione on flutamide-induced lipid peroxidation and changes in cholesterol content using common laboratory markers. *FABAD J Pharm Sci* 2008;33:1-10.
  32. Slaoui M, Fiette L. Histopathology procedures: From tissue sampling to histopathological evaluation. In: Gautier JC, editor. *Drug Safety Evaluation: Methods and Protocols*. Methods in Molecular Biology-Springer; 2011. p. 691, 69-71.
  33. Brahm J, Brahm M, Segovia R, Latorre R, Zapata R, Ponichik J, *et al.* Acute and fulminant hepatitis induced by flutamide: Case series report and review of the literature. *Ann Hepatol* 2011;10:93-8.
  34. Giorgetti R, di Muzio M, Giorgetti A, Girolami D, Borgia L, Tagliabracci A, *et al.* Flutamide-induced hepatotoxicity: Ethical and scientific issues. *Eur Rev Med Pharmacol Sci* 2017;21:69-77.
  35. Teppner M, Böss F, Ernst B, Pähler A. Application of lipid peroxidation products as biomarkers for flutamide-induced oxidative stress *in vitro*. *Toxicol Lett* 2015;238:53-9.
  36. Legendre A, Jacques S, Dumont F, Cotton J, Paullier P, Fleury MJ, *et al.* Investigation of the hepatotoxicity of flutamide: Pro-survival/apoptotic and necrotic switch in primary rat hepatocytes characterized by metabolic and transcriptomic profiles in microfluidic liver biochips. *Toxicol In Vitro* 2014;28:1075-87.
  37. Fouad AA, Al-Mulhim AS, Jresat I. Therapeutic effect of coenzyme Q10 against experimentally-induced hepatocellular carcinoma in rats. *Environ Toxicol Pharmacol* 2013; 35:100-8.
  38. Mirmalek SA, Boushehrinejad AG, Yavari H, Kardeh B, Parsa Y, Salimi-Tabatabaee SA, *et al.* Antioxidant and anti-inflammatory effects of coenzyme Q10 on L-arginine-induced acute pancreatitis in rat. *Oxid Med Cell Longev* 2016; 2016:5818479.

**Financial Support:** None; **Conflict of Interest:** None

**How to cite this article:** Adikwu E, Ebinyo NC, Aagbadabina H. Coenzyme Q 10 Abrogates Flutamide-Induced Hepatotoxicity in Albino Rats. *J Med Sci Health* 2019;5(2):1-8.

Date of submission: 06-05-2019

Date of review: 13-05-2019

Date of acceptance: 18-05-2019

Date of publication: 10-10-2019